in *Phaseolus lunatus* seed and in flax seed. This enzyme is of the emulsin type (*i.e.*, it appears to hydrolyse β-glucosides) and exhibits similar activities, but it also presents certain well-marked differences from emulsin, which will be the subject of further investigation.

Cyanogenesis in Plants.

Part V.—*The Occurrence of Phaseolunatin in Cassava* (Manihot Aipi *and* Manihot utilissima).

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(Received April 11,—Read May 10, 1906.)

The "sweet" and "bitter" cassava plants are indigenous to Southern and Central America, whence they have been introduced, especially the sweet variety, into almost all tropical countries and are now widely cultivated for the sake of their edible starchy roots, which are used for the manufacture of the various forms of cassava starch, of which tapioca is perhaps the best known.

The plants are known in their native habitat by a variety of vernacular names of which only one, "manioc" or "mandioca," has come into general use. The name "cassava" seems to be restricted in South America to the flour or meal made from the roots, but outside South America this name has come to be applied to the whole plant.

There are many varieties of cassava plants in cultivation in the tropics, but these all appear to belong to either the "bitter" or "sweet" forms. These two forms were regarded by Pohl* as distinct species and were named by him *Manihot utilissima* and *Manihot Aipi* respectively.

By other botanists the "sweet" cassava is regarded as a variety or perhaps a cultivated race of *Manihot utilissima*,† whilst others take the view that Pohl's *Manihot Aipi* is identical with *Manihot palmata*.‡ Colonel Prain, Director of the Royal Gardens, Kew, whom we have consulted on this point, is of opinion that on the evidence at present available, Pohl's view,

* 'Pl. Bras. Ic.,' i, vol. 32, p. 24.

† Compare Sagot, 'Bull. Soc. Bot. France,' 1872, vol. 18, p. 341.

‡ 'Index Kewensis,' fasc. iii, p. 162; and Peckolt, 'Pharm. Rund.,' 1886, vol. 4, p. 57.

that the plants yielding the "bitter" and "sweet" cassavas are distinct species, is most likely to be the correct one.

The poisonous properties of the bitter manioc root seem to have been known to the natives of Central and Southern America from very early times and the process they use for preparing an edible meal from it seems to be designed with a view to the complete elimination of the poison. The process is described by Sagot* as follows: "The roots are scraped, peeled and washed. This clean material is then rasped and the pulp left to ferment for 24 hours. It is then placed in a long flexible basket made of plaited rushes. This is suspended by a handle at its open end and to the other a heavy weight is attached by which means the pulp is compressed and a highly poisonous juice oozes through the plaits. The pressed meal is taken out and exposed for some time over a fire and then pounded, coarsely sifted, and again exposed on a brass plate over a fire; during this operation the meal is constantly stirred so that it assumes a granular form."

The same author states that: "Sweet cassava contains so small a quantity of poisonous matter that the roots are cooked at a fire and eaten like potatoes."

Probably the earliest reference in European literature to the poison contained in cassava root is that made by Clusius,† who says: "Caçavi autem panis est quo Indi tot seculis vitam sustentarunt et hodie etiam vescuntur nostri Hispani.

"Nec minore admiratione dignum est omnem Yucam‡ in continenti nascentem tametsi quæ ad S. Dominicum nascitur (ex qua Caçavi fit) similem salutarem esse, ejusque fructum (radicem) edulem et succum inde manantem potabilem, nullamque noxam adferre; eam autem quæ ad S. Dominicum provenit (quacumque tandem ratione edatur) ejusque succum non coctum, perimere. Locorum vero naturam tanti momenti esse ut quod salubre alimentum in continenti præbet, id in omnibus insulis præsens fit venenum. Quemadmodum Columella de Persico scribit, perniciosum venenum in Perside fuisse, at ubi in Italiam translatum fuit noxium illum succum deposuisse et suavem salutaremque præbuisse."

This early observation of Clusius, that the generation of a poison in cassava is associated with the conditions under which it is grown, is of special interest in view of Wiley's statement that cassava cultivated in subtropical countries, as distinct from the tropics, becomes much less toxic.§

* *Loc. cit.*

† 'Liber Exoticorum' (Leyden, 1605), lib. 10, fol. 339.

‡ Yuca is one of the vernacular names of the cassava plants and was in common use at that time in the Spanish South American Colonies.

§ 'Bull. U.S.A. Dept. Agric.,' Div. Chem., No. 44.

The first observation as to the volatile nature of the poison contained in the roots seems to have been made by Fermin,* who obtained from 50 lbs. of manioc juice 3 ounces of an intensely poisonous distillate, 35 drops of which were sufficient to poison in a few seconds a condemned slave to whom they were administered. In 1828, according to Henry and Boutron-Charlard,† Soubeiran and Pelletier endeavoured to isolate the toxic principle of cassava, but were unsuccessful. In 1833 Henry conducted a similar investigation, but owing to lack of material was unable to obtain any definite results. The same author, in association with Boutron-Charlard, took up the matter again in 1836,‡ and succeeded in identifying the poisonous volatile constituent of cassava with hydrocyanic acid.

It is worth noting in this connection that although Scheele had discovered hydrocyanic acid in 1780, he was apparently unaware that it was poisonous. The toxicity of the acid was first established by Henry and Boutron-Charlard in 1833. These authors made a complete analysis of cassava, and found that the roots contained starch, free hydrocyanic acid, sugar, an organic magnesium salt, a bitter principle, a fat, a nitrogenous substance, calcium phosphate, and woody fibre. They also carried out a number of physiological experiments with fowls, and found that the distillate prepared from cassava invariably produced fatal results when administered.

The first trustworthy estimations of the amounts of hydrocyanic acid obtainable from cassava were made by Francis,§ who established the important fact that, as regards the cassava roots grown in the West Indies, both the "sweet" and "bitter" varieties yield about the same quantities of hydrocyanic acid.

Francis' observations as to the occurrence of hydrocyanic acid in the "sweet" as well as in the "bitter" cassava grown in the West Indies were confirmed by Carmody,‖ who concluded that the principal difference between the two plants is that in the "bitter" cassava the acid is uniformly distributed throughout the root, whereas in the "sweet" cassava it is located principally in the rind of the root. Carmody also observed that in sweet cassava the acid occurs partly free and partly combined.

In 1886 Peckolt¶ examined a number of the principal varieties of cassava grown in Brazil, and showed that most of these, both the "sweet" and

* 'Mém. Acad. Sci. Berlin,' 1764.
† 'Mém. Acad. Royale de Médecine,' Paris, 1836, vol. 5, p. 212.
‡ *Loc. cit.*
§ 'Analyst,' 1870, vol. 2, p. 4.
‖ 'Lancet,' 1900,
¶ 'Pharm. Rund.,' 1886, vol. 4, p. 227.

"bitter" forms, yielded prussic acid. This investigator made a careful search for amygdalin, but was unable to obtain evidence of the existence of this or any similar glucoside in the roots. Peckolt obtained a number of substances from cassava; these were ill-defined bodies with the exception of manihotin and manihotoxin, which were crystalline. The former is described as melting at 160°, and somewhat resembling mannitol. It does not contain nitrogen.

Peckolt also stated that hydrocyanic acid does not exist in the roots until these are withdrawn from the soil, and suggested that the acid is formed as the result of atmospheric action.

That the hydrocyanic acid does not wholly occur free in the leaves of the cassava plant was observed by van Romburgh,* who, by macerating the leaves in water, and distilling the resulting liquid, obtained a distillate containing both acetone and hydrocyanic acid, and suggested that the hydrocyanic acid occurred partly combined with acetone and partly in the form of a glucoside.

Preliminary Experiments.

The results of previous investigators on the whole tended to show either that hydrocyanic acid existed in a free state in cassava roots, or that if it were present in the form of a glucoside, the latter must be of a very unstable character, and readily decomposed with the liberation of hydrocyanic acid.

With a view to avoiding any risk of decomposition of the glucoside, we endeavoured at first to import fresh cassava roots from the West Indies, but it was found to be impossible to do this successfully, since the roots decomposed to a considerable extent in transit.

Recourse was then had to "bitter" cassava root, sliced in a fresh state and dried in the sun, and it was found that by this means material fairly rich in a cyanogenetic substance could be obtained. By preliminary experiments with this material it was found that the rind of the bitter root was much richer than the interior portion, and recently we have worked only with the rind prepared by stripping it from the fresh root of bitter cassava and drying it in the sun.

The whole of the material used in the present investigation has been obtained from the West Indies, and we are indebted to Sir D. Morris, Imperial Commissioner for Agriculture in the West Indies, who enabled us to obtain supplies in the first instance, and to Mr. Bovell, Superintendent of the Botanic Station, Barbados, who kindly undertook the preparation

* 'Annales du Jardin Botanique de Buitenzorg,' 1899, ii, vol. 16, p. 15.

of the various consignments of dried bitter cassava root and rind we have received.

Isolation of the Glucoside.

Estimations of the amounts of hydrocyanic acid obtainable from the dried sliced root and from the dried rind of bitter cassava were made by the method we have generally used for this purpose, viz., the complete extraction of the ground material with 90 per cent. alcohol and the hydrolysis of the glucoside contained in the residue left after distilling off the alcohol from this extract. This hydrolysis was accomplished by dissolving the residue in water and distilling the liquid almost to dryness after the addition of a few cubic centimetres of hydrochloric acid, the hydrocyanic acid in the distillate being titrated with silver nitrate solution by Liebig's method.* In this way it was ascertained that the dried sliced root yielded about 0·009 per cent. and the dried rind of the bitter cassava 0·035 per cent. of acid. These results agree fairly well with the quantities of the acid found by Francis, but the last is somewhat higher than those recorded by Carmody,† which ranged from 0·0113 to 0·0238 per cent. Owing to the impossibility of importing fresh roots, we have not been able to make any useful determination of the amount of acid obtainable from fresh roots of "sweet" and "bitter" cassava or from different parts of such roots, but we understand that investigations of this kind are now being carried out in India by Dr. J. W. Leather, Government Agricultural Chemist.‡

For the isolation of the glucoside the finely-ground cassava rind was completely extracted by percolation with 90 per cent. of alcohol. The solvent was distilled from the extract, the syrupy residue slightly diluted with water, filtered from the precipitated resinous and oily matters, and the filtrate decolorised by adding lead acetate, filtering out the precipitated lead compound of the colouring matter, and removing the excess of lead from the filtrate by treatment with sulphuretted hydrogen. This purified extract was then evaporated almost to dryness under reduced pressure at the ordinary temperature. The light brown syrup so obtained showed no tendency to crystallise even after long standing. It was therefore dissolved in alcohol and the solution poured into excess of ether. The matter precipitated by the ether consisted principally of dextrose. The decanted liquid was again evaporated nearly to dryness, the residue dissolved in alcohol, and the precipitation with excess of ether repeated. After each operation the

* Compare 'Phil. Trans.,' 1901, B, vol. 194, p. 515; 1902, A, vol. 199, p. 399; 'Roy. Soc. Proc.,' 1904, vol. 72, p. 285.

† *Loc. cit.*

‡ Annual Report of the Imperial Department of Agriculture, 1905.

solution was evaporated to dryness and the residue set aside so that crystallisation could occur, but it was only after this tedious process of dissolution in alcohol, precipitation by excess of ether, and evaporation to a syrup had been repeated five times that a crystallisable residue was obtained.

The difficulty of separating the glucoside from these residues appears to be due to the saccharine and extractive matters present, and it is only by the practically complete removal of these impurities by precipitation from alcoholic solution with excess of ether that the glucoside can be induced to crystallise. This process is both tedious and wasteful, the greater part of the glucoside being included in the several uncrystallisable fractions obtained when the solutions in alcohol are poured into excess of ether.

The crystalline residue eventually obtained was recrystallised from alcohol until colourless and of constant melting point. It crystallised in the spreading rosettes of colourless needles which are characteristic of phaseolunatin, had the same cool, bitter taste, and, like it, was readily soluble in water, less so in alcohol, and almost insoluble in dry ether. It melted at 138° (corr.). A mixture of phaseolunatin, prepared from the seeds of *Phaseolus lunatus*, and the cassava glucoside also melts at this temperature. There can be no doubt, therefore, that the glucoside of cassava is identical with phaseolunatin, which we have shown to be a dextrose ether of acetone cyanhydrin.

Hydrolytic Products of the Cassava Glucoside.

A portion of the purified extract, prepared as already described, was dissolved in water, a few cubic centimetres of 10-per-cent. hydrochloric acid added, and the mixture distilled almost to dryness. The distillate gave the Prussian blue reaction readily. To the remainder of the distillate, freshly-prepared lead hydroxide was added, and the mixture allowed to stand for some time, so that the hydrocyanic acid might be removed as lead cyanide. The filtrate from this was redistilled, and the first few cubic centimetres collected. To this were added a few drops of benzaldehyde, a similar small quantity of potassium hydroxide solution, and enough alcohol to dissolve the benzaldehyde added. On standing, the liquid deposited the characteristic crystals of dibenzylideneacetone (melting point 112°). The volatile hydrolytic products of the glucoside of cassava are, therefore, acetone and hydrocyanic acid, identical with those of phaseolunatin, affording further proof of the identity of the cassava glucoside with phaseolunatin.

The Enzyme of Cassava Root.

The enzyme was prepared in the usual way by extracting the ground dried rind of bitter cassava root with water previously saturated with chloroform,

This liquid, when poured into an excess of alcohol, yielded a white precipitate of proteid matter, which, when dried by exposure to air on glass plates, formed slightly brown, granular masses. This preparation readily decomposed aqueous solutions of amygdalin and salicin, and also of phaseolunatin prepared from the seeds of *Phaseolus lunatus.*

The enzyme contained in the roots of the bitter cassava evidently closely resembles, and is probably identical with, the emulsin-like ferment obtained by us from the seeds of *Phaseolus lunatus,** and also from young flax plants (see this series, Part IV).

Cassava, therefore, like the other plants producing prussic acid which we have examined in the course of this investigation, contains a cyanogenetic glucoside, together with an enzyme capable of decomposing it. It is remarkable that the same glucoside, phaseolunatin, should occur in such different plants as *Phaseolus lunatus, Linum usitatissimum*, and *Manihot* species.

Although, for the reasons stated, we have found it convenient to employ the root of the bitter cassava for the isolation of the glucoside and its identification with phaseolunatin, there can be little doubt that this same glucoside occurs in sweet cassava, and that it is responsible for the production of prussic acid in that plant.

* 'Roy. Soc. Proc,' 1904, vol. 72, p. 285.